WILDLIFE TOXICOLOGY and POPULATION MODELING

Integrated Studies of Agroecosystems

Edited by

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Comparative Avian Toxicology: What Is Its Role in Predicting and Monitoring the Effects of Agricultural Pesticides

Edward W. Schafer, Jr.

ABSTRACT

Determining the comparative toxicology of pesticides and other chemical agents for wild bird species (quail, ducks, etc.) is an emerging area of environmental concern. In North America, excluding Central America and Mexico, over 700 wild bird species have been identified as common or occasional residents or visitors. Nevertheless, toxicological effects of most agricultural, industrial, or environmental chemicals are poorly characterized for almost all of these species. In fact, in-depth comparative toxicological studies of chemicals have been reported on less than ten wild bird species (including game birds). These species represent about 1% of all North American species. Where toxicological characterization has occurred, generally it has been limited to testing specific pesticides or groups of pesticides (organophosphates, carbamates, organochlorines, etc.) and not the broader array of potentially or purposefully introduced compounds of environmental concern. Additional research designed to better define the effects of these chemicals on live, intact birds and other warm-blooded animals is unlikely in the future due to increasing restrictions on the use of animals in research. Thus, wildlife toxicologists will have to make the most of previously gathered data and hone their predictive skills. This task will not be easy, and may, for some chemicals and many bird species, be impossible.

Besides these factors, specific bird species of concern to regulatory agencies and industry are usually limited in numbers or availability. In order to provide a means of assessing risk for species of limited availability, regulators and wildlife toxicologists have been seeking ways of extrapolating laboratory test results using available or abundant species, to other species, and/or to field situations. The concepts of surrogate, indicator, and sentinel species have been developed and used with limited success in the field of wildlife toxicology. Variations in chemical intoxication among and within family, genus, and species are large and chemical effects vary widely; associated environmental parameters also vary in quality and quantity. Given the current state of laboratory data and the status of existing databases that can be used to predict laboratory effects, predictions describing potential effects on untested species will have to be tempered by many qualifiers.

In the field, the situation is more complex because available databases are limited and inaccurate. Developing accurate data in the field is difficult and expensive even for a single bird species in a well-defined geographical area with a single chemical. The

difficulty of performing a similar study involving several species and multiple sites is magnified many times by orders of magnitude. These difficulties can be overcome by persistence and innovative thinking on the part of regulators and industry. Close cooperation toward meeting common goals is an essential part of this effort. The lead taken by the Avian Effects Dialogue Group and other industry- and regulator-sponsored groups should result in advances in the knowledge and understanding of the complex issues currently facing wildlife toxicologists.

KEY WORDS

toxicology, pesticide, bird, monitoring, prediction

INTRODUCTION

North American Bird Populations

Approximately 650 species of wild birds are known to breed on the North American continent (excluding Mexico and Central America), and another 50 species are regular or occasional visitors.[1] The total of 700 species represents 75 bird families. Estimates of the total number of birds in North America at any given time vary considerably according to seasonal factors; however, although peak numbers are inaccurately known, they are often given as about 20 billion,[1] roughly 50 to 60 birds per human inhabitant. Estimated populations of up to 200 million waterfowl (ducks and geese) and 800 million upland game birds (pheasant, quail, etc.) are present in North America.[2,3] The waterfowl and gamebird species encompass five separate families but probably constitute no more than 5% of the avian populations of North America. The passerines (27 families) probably constitute more than 50% of the total postbreeding North American bird population.[4]

The number of individual birds within a given species or family vary considerably. Some species that are considered in danger of extinction may number less than 100 individuals, whereas an abundant species such as the European starling (*Sturnus vulgaris*) may number as many as 200 to 300 million individuals shortly after the breeding season.[5] Within passerines, the subfamily Icterinae (blackbirds and orioles) probably numbers about 2 billion in the postbreeding season, or up to 10% of the total North American population of birds.[2,5] Of the 13 most abundant species in North America, 5 are Icterines: the red-winged blackbird (*Agelaius phoniceus*), Western meadowlark (*Sturnella neglecta*), common grackle (*Quiscalus quiscala*), Eastern meadowlark (*Sturnella magna*), and brownheaded cowbird (*Molothrus ater*).[6] Other numerous passerines, besides starlings, are the house sparrow (*Passer domesticus*), horned lark (*Eremophila alpestris*), American robin (*Turdis migratorius*), common crow (*Corvus brachyrhynchos*), barn swallow (*Hirundo rustica*), and cliff swallow (*Hirundo pyrrhonota*).[6] Thus, of the 13 most abundant North American species, 12 are passerines. Only one other order, Columbiformes, contains a species on the most abundant list, the mourning dove (*Zenaida macroura*).[6] The northern bobwhite (*Colinus virginianus*), order Galliformes, ranks in the top 20 species, and the mallard (*Anas platyrhynchos*), order Anseriformes, in the top 50 species.[2]

Potential Exposure

All species of birds that inhabit or visit North America are exposed to agricultural, industrial, or other introduced chemicals, as well as naturally occurring chemicals in their food, water, and air. Birds are also ubiquitous throughout North America, feeding and breeding in a diversity of habitats year-round or seasonally. They are relatively large, numerous, readily observable, and usually identifiable as individuals. Birds are also aesthetically and economically important to the human population of North America. Over 125 million Americans are involved in observing or harvesting these animals each year.[7] For these reasons and many others, measuring the numbers, diversity, and incidental mortality of these species has been proposed as a means of monitoring or predicting environmental health, adverse environmental impacts, and species survivability, particularly as it relates to introduced chemical agents, mostly pesticides.[8,9] A number of specific concepts have been proposed to monitor or predict risk. Through 1990, these concepts have been elucidated at meetings of the Avian Effects Dialogue Group, a group convened by The Conservation Foundation at the request of industry and the U.S. Environmental Protection Agency (EPA) to provide guidance in the assessment of pesticide risk to birds.[10]

Species Concepts

The concepts of surrogate, indicator, and sentinel species have been used or described in various ways to allow for the testing or monitoring of small numbers of birds or bird species, and to extrapolate the observed effects to other species in the avian community or to other effects with some degree of confidence.[4,10–13] The definitions that follow delineate these concepts for purposes of discussion.

Surrogate species is an alternative species selected to represent a single species of concern. The surrogate species is usually selected because the species of concern is not readily available for study due to its small number, isolated distribution, legal status, or the physical damage that could occur during capture or holding. Based on physiological and behavioral parameters, the surrogate is assumed to represent the species of concern, to which it normally has close taxonomic associations. For use in the laboratory, a surrogate species must be tractable and available in numbers either through captive breeding or wild-capture techniques. In addition, surrogates used in the field should be readily observable and occupy a similar ecological niche as the species of concern. Thus, the response of the surrogate to applied chemical stress is assumed to be similar in the laboratory and field within a degree of accuracy that is acceptable to the investigator. This definition of surrogate species is more limited than is commonly used in many ecotoxicological studies.

Indicator species refers to a species selected to represent a larger group of species of concern. The indicator species is usually selected because those species of concern are too numerous to study individually, are not readily available for study, or cannot be studied without physical damage. The types of similarities to the species of concern often depend upon the question being posed by the study; physiological, behavioral, or taxonomic similarities may not all be necessary. Primary factors for the use of indicator species are often economics and availability. In the laboratory, an indicator species must be tractable and available in numbers through captive breeding or wild-capture techniques. Similar constraints exist for indicator species used in the field. In addition, the indicator species should have similar exposure potential and occupy a similar ecological niche as the species of concern. Responses of indicator species to applied chemical stress in the laboratory and the field are assumed to be similar to the species of concern; however, the ability to extrapolate results is often limited by the size and composition of the group that contains the species of concern. Indicator species are often proposed for study because of their potential to validate generalized laboratory studies. This definition of indicator species can be broadly applied, but contrary to its use in many ecological studies, its use in this case does not imply ecological monitoring.

Sentinel species is defined as a species of interest selected as a signal of impending potential harm or change to other species or the environment. The sentinel species is normally selected because of its availability, observability, and sensitivity to the toxicological issue of concern. The taxonomic similarity of the sentinel to other species of concern is often irrelevant, as is its similarity of exposure. In most cases, sentinel species are the most toxicologically sensitive and pose the greatest risk of exposure. Sentinel species are not used often in the laboratory investigation of the comparative effects of chemicals on birds, but are generally limited to field use. They may either occur naturally or be specifically placed on site; such species should be readily observable.

The scope of biological extrapolation among birds can be narrow (within a family) or broad (among families or across several orders). It can extend from confined laboratory studies to simulated field or pen studies, field investigations, and often monitoring activities. The scope of toxicological extrapolation can be equally diverse, ranging from acute, subacute, or chronic toxicity, to gross effects on reproduction, to actual or projected effects on individuals or populations.[9] Thus, risk assessment is an immense undertaking, but it has very important and long-term rewards if it can be reliably accomplished. Federal, state, and local government agencies, universities, private industry, and the general public all have a stake in this activity. After decades of study and discussion, the future direction and application of these concepts seem to be slowly progressing.

History

The domestic chicken (*Gallus domesticus*) was probably the first bird routinely used as an indicator for the effects of chemicals on other birds, primarily because of its availability, known husbandry requirements, and economics. It was used initially to determine the acute toxicity of chemicals to birds and continues to be used today to evaluate the veterinary importance of animal drugs for poultry. Finches, canaries, or other small songbirds have been used by miners for hundreds of years as sentinels to detect the presence of toxic gases in mines.

In the 1940s and 1950s, the northern bobwhite and the ring-necked pheasant (*Phasianus colchicus*) replaced the chicken as indicators of bird toxicity, as researchers assumed the responses of these semidomesticated wild birds were more closely related to those of other gallinaceous gamebirds than were those of the chicken. In the 1960s, the coturnix (*Coturnix coturnix*) and the mallard were added to the test species commonly used in the laboratory as indicators of avian toxicity.[4]

In the 1970s, four more species were added to the list of avian test subjects for which relatively large databases had accumulated: the European starling, red-winged blackbird, house sparrow, and house finch (*Carpodacus mexicanis*).[14-16] Since then, no significant published additions have been made to avian toxicology databases, although additional information has accumulated in the files of the U.S. EPA. Pesticide producers have contributed to this database by meeting the data requirements specified by the EPA under its regulatory authority over the registration and reregistration of pesticides. Primary EPA test species are northern bobwhite and mallard, which are used as indicator species of general avian toxicity in order to predict risks of environmental concern. Most other avian toxicology databases that have been assembled are specific to individual species or chemicals, or combine data from numerous sources and methods.

Comparative Avian Toxicology

Published studies covering the comparative acute toxicity of chemicals to birds are limited but available. Tucker and Haegele[17] summarized their laboratory studies, designed to determine whether the indicator species concept was valid, using 6 bird species (from 4 families) and 16 pesticides.[17] By using a ranking procedure, they showed that no significant differences in sensitivity existed among the species tested, but that large variation existed among species with respect to an individual pesticide. However, there was a significant difference in sensitivity between the chukar partridge (*Alectoris chukar*) and the house sparrow near the $p = 0.05$ level. As a result of their study, the authors concluded that if it was necessary to know the toxicity of a specific pesticide on a bird species, then ". . . the species itself should be used . . . to avoid the need for extrapolation." Additional data from these authors and Hudson et al.[18] are also available in a handbook describing the acute effects of a large number of pesticides to many additional animal species.

In a 1967 study, data gathered by this author and others suggested that red-winged blackbirds were more sensitive than starlings to intoxication from 22 carbamates.[14] In 1972, using 7 species covering 4 families and 148 chemicals from a variety of sources, it was concluded that the ring-necked pheasant was significantly less sensitive than other species, and the house finch and the red-winged blackbird were significantly more sensitive than the remaining species, including mallards and pheasants.[15] Also in 1972, the results of comparative testing of 369 chemicals showed that red-winged blackbirds were significantly more sensitive to intoxication than starlings.[16] In 1973 a significant correlation between the oral or dermal acute toxicity of 17 chemicals to red-winged blackbirds, house sparrows, and red-billed quelea (*Quelea quelea*) was demonstrated.[19] In 1979, a study involving the toxicity of 36 pesticides from 3 different classes on 6 bird species covering 4 families showed that starlings were significantly less sensitive to the pesticides than the remaining 5 species. Again, red-winged blackbirds and sparrows were the most sensitive species tested. However, the authors concluded that ". . . the basis for the present use of indicator bird species for estimating or predicting hazards to other species of wild birds is not well established".[4] At the Second Annual Meeting of the Society of Environmental Toxicology and Chemistry (SETAC) in 1981, a presentation described the acute toxicity of 63 monosubstituted anilines and pyridines to 4 bird species (2 families) and 2 species of mammals.[20] This presentation, while not published outside the proceedings, showed that although generalized structure activity relationships exist for many of the tested chemicals, there is considerable variation between species. Definitive structure activity relationships between species were also questioned. In 1982, a comparative study of the acute toxicity and repellency of three chemicals to five African bird species (same family) showed that toxicological responses were similar only within individual chemicals.[21] In 1983 an acute toxicity study involving 130 chemicals and 3 species representing 3 families of birds concluded that red-winged blackbirds were significantly more sensitive than coturnix and starlings, and that the latter were not different.[22] Repellency (a physiological or behavioral measure) was not correlated with toxicity.

Only a limited number of studies have been reported on the comparative subacute intoxication of birds by chemical agents. Two early papers discussed the relationships between four species of young birds representing two families. Toxicological sensitivity was correlated with body size and chemical structure, but not species. However, the authors reported that "inconsistencies in the relative sensitivity of the four species . . . suggest that other species would be much more sensitive to different chemicals".[23,24] In a third related study, four pesticides were tested on four species (two families) and showed that passerines were considerably more sensitive to intoxication than members of Galliformes. This sensitivity was also thought to be a function of body weight.[25]

DISCUSSION

Literature sources indicate that it is not unusual for toxicity levels to differ by two to three orders of magnitude among orders of animals. Particularly true when data are collected from a number of unrelated sources, such differences can be due to test methodology; variations among family, species, and individual responses to chemical agents; and data interpretation.[26] Thus, although most environmental toxicologists agree on the need for concepts that will allow for the broad extrapolation of data to assist in providing accurate risk assessments, there are questions as to how accurately these data can be predicted.

In order to graphically illustrate the multitude of problems that occur when one tries to extrapolate data based on generalizations to specific chemicals or species, the data on acute avian toxicity collected by the Denver Wildlife Research Center was re-examined. These data are characterized by a broad spectrum of chemicals (more than 2500) and involve 45 species of birds from 15 families. It was gathered by the same individuals over a 20-year period using similar methods and the same facility. From this set of data, information was extracted for chemicals with acute toxicity data on at least ten bird species covering at least five families.[4,18,22] Where more than one data point (LD_{50}) was available for the same species, the lowest (most toxic) value for physically mature birds was used. Three specific areas not covered by other authors were targeted: toxicity distribution for individual chemicals among species or families (i.e., what is the range and distribution of toxicity for a given chemical); the location of recognized indicator, surrogate, or sentinel species on the toxicity spectrums of selected chemicals (i.e., how well do these species predict toxicity on a chemical-by-chemical basis); and the toxicity distribution among species or families of closely related chemical analogues (i.e., how well can data from a given chemical be used to predict toxicity for a closely related chemical across species or families).

Species and Family Toxicity Distribution

Figures 1 through 5 show the toxicity distributions for five chemicals representing four different chemical classes. They are examples of the species/family distribution of toxicity as well as the magnitude variation encountered in such a distribution. The figures display the information for both species and families for which the toxicity values (LD_{50}) fall within an artificially established order of magnitude range (i.e., 1 to 10 mg/kg, 11 to 100 mg/kg, etc.). The chemicals shown are 4-aminopyridine (Figure 1), aprocarb (Figure 2), fenthion (Figure 3), methiocarb (Figure 4), and Starlicide (Figure 5). These figures show that all of the toxicity distributions for five chemicals ranged over two to three orders of magnitude. This information is similar to that elucidated by Tucker and Leitzke[26] and shows the tremendous range of values that can occur even when using data sets with limited variables. Although the actual ranges of toxicity are overstated by this presentation, Figure 6 shows that the actual minimum range (represented by 4-aminopyridine) is slightly more than a single order of magni-

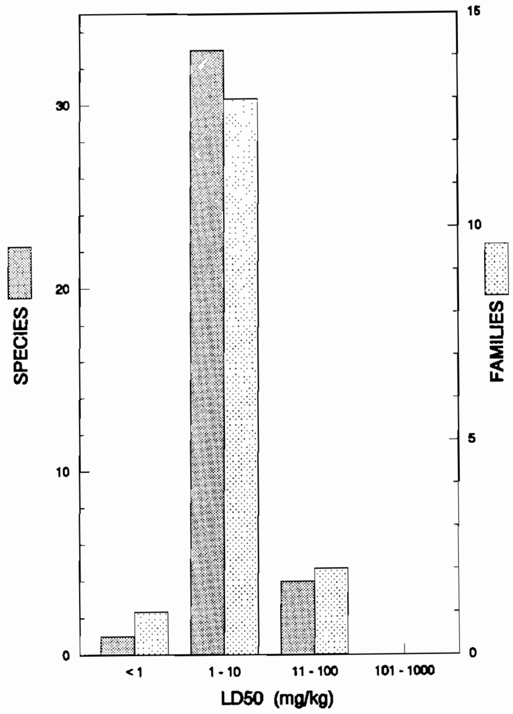

FIGURE 1. Acute oral toxicity of 4-aminopyridine to 13 families of birds.

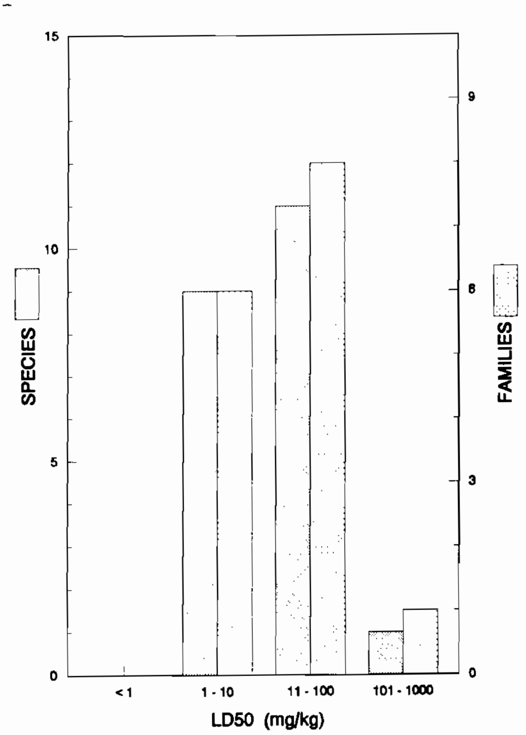

FIGURE 2. Acute oral toxicity of aprocarb to 11 families of birds.

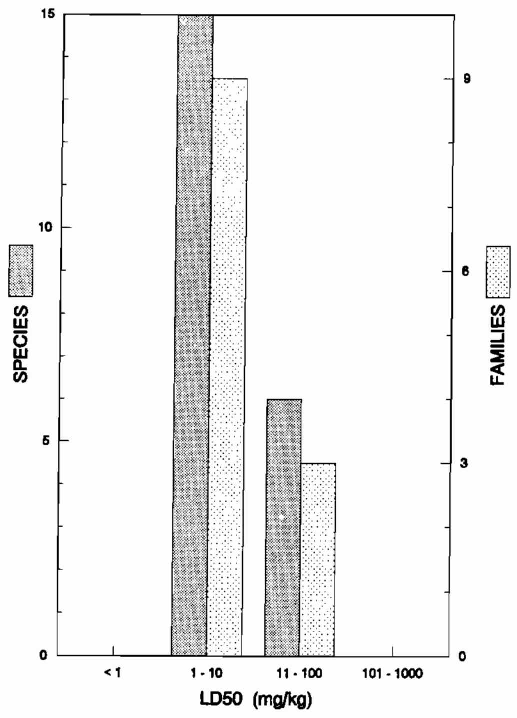

FIGURE 3. Acute oral toxicity of fenthion to 10 families of birds.

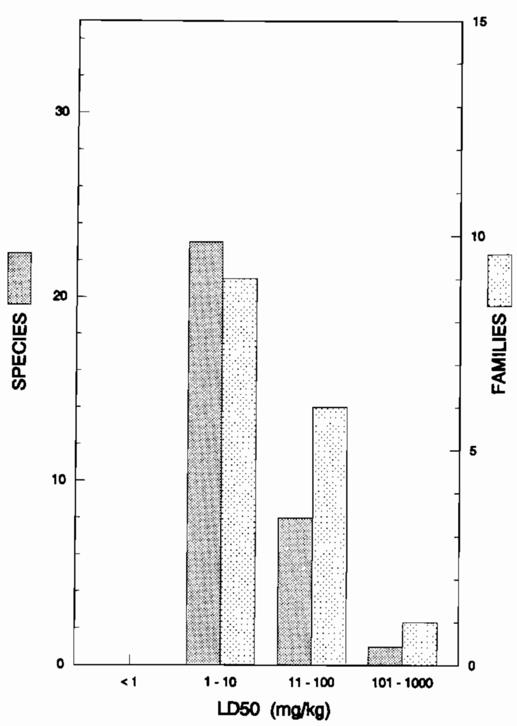

FIGURE 4. Acute oral toxicity of methiocarb to 13 families of birds.

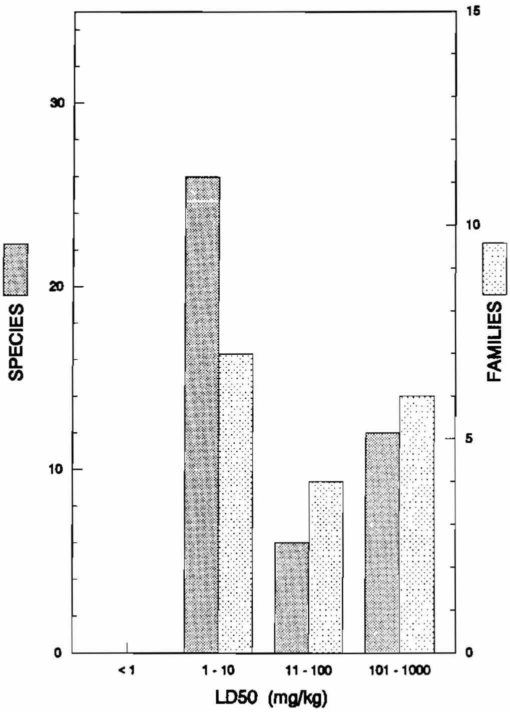

FIGURE 5. Acute oral toxicity of Starlicide to 15 families of birds.

tude, and the maximum (Starlicide) is very close to three orders of magnitude. Accepting such variation and applying those data to risk assessments could therefore result in either over- or underestimating risk by factors of up to 1000. Such estimation errors are not trivial, even if extrapolation factors are used in the risk assessment process to accommodate such variation.

Data associated with Figures 1 through 5 provide good examples of the differences between bird responses to different chemicals. The chemicals represented by these data are unique in that a single source existed for data on more than ten species of wild birds. Most pesticides have similar data for only two to five species, and often those data are generated by more than one laboratory.

Predictions Based on Sensitivity

Environmental toxicologists have been concerned about doing a better job of assessing risk by producing data on specific species that are very sensitive or very insensitive to chemical intoxication. Using moderately small to large data sets covering a number of chemicals and species, researchers have shown that some species exhibited a predictable sensitivity to chemical intoxication, and that such predictability could be used for risk assessments of specific chemicals. Such broad-scale generalizations assume, however, that predictability is not related in any meaningful way to chemical structure. They further assume that the structures represented in the sample used to establish a significant effect also represent other nontested chemical structures.

The fallacy of these assumptions can be demonstrated by examining Figure 6. This figure displays a comparison of the toxicity ranges for the five chemicals covered in the first five figures plus two additional chemicals for which bird toxicity data on at least

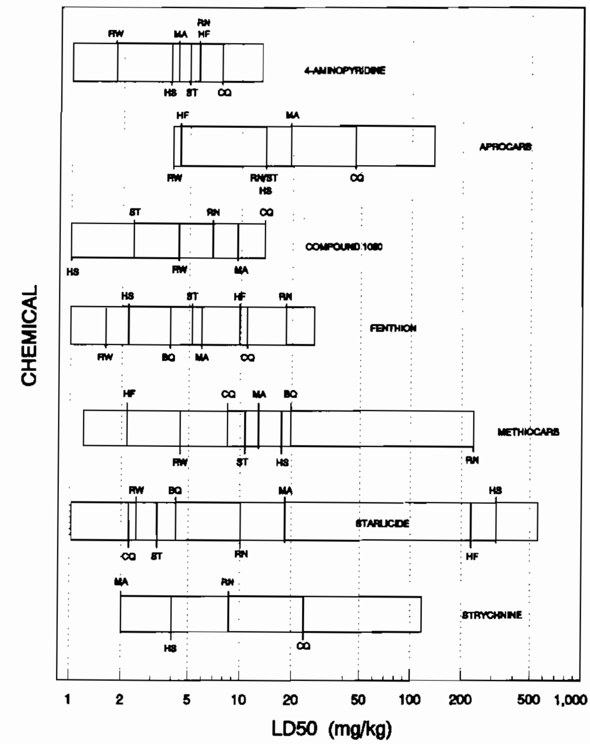

FIGURE 6. Location of selected species on a bar representing the toxicity range for seven chemicals. (See text for species abbreviations.)

ten species were available. The bar for each chemical represents the defined avian toxicity range for that chemical; lines and corresponding initials show the locations in this range of the toxicity values for eight avian species most often used as indicator species in North America. The red-winged blackbird (RW), house sparrow (HS), and house finch (HF) generally have been considered "sensitive" species, and the starling (ST), ring-necked pheasant (RN), and coturnix (CQ) as relatively "insensitive" species. The northern bobwhite (BQ) and mallard (MA), used in most EPA-mandated tests as indicators of avian toxicity, are intermediate in their sensitivity. Careful examination of the data for each chemical does not show a single case in which the three "sensitive" species are all more susceptible to intoxication than the four "insensitive" species. In fact, considerable overlap exists, and in some cases an "insensitive" species is the most impacted and a "sensitive" species is the least impacted. Not only is there a lack of pattern on a chemical by chemical basis, but the pattern changes dramatically among chemicals. If these data are representative of other potential data sets for other pesticides or industrial chemicals, it would be extremely difficult to justify the use of any one species as a surrogate for another or as an indicator of a larger group (e.g., coturnix for gallinaceous birds, red-winged blackbirds for passerines). Also, it would be difficult to select a sentinel species of known high sensitivity to chemical intoxication that can serve as a warning of impending chemical harm. The only way that this could be effectively accomplished is to accept that whatever predictions were going to be made could be off by two to three orders of magnitude in either direction. Thus, although general predictions can be made over large numbers of chemicals and a variety of chemical classes, if one examines the application of those predictions to individual chemicals, they do not hold up.

Predictions Based on Chemical Grouping

Figure 7 illustrates another data set covering 21 organophosphates, 9 carbamates, and 4 organochlorine pesticides that were tested by a single laboratory and investigator using 6 bird species, then ranked by order of sensitivity. Four of these species were included in the discussion of Figure 6, two being "sensitive" (red-winged blackbird and house sparrow) and two being "insensitive" (starling and coturnix). When these data were analyzed over all 35 chemicals (one chemical was tested that did not fall into any of the three classes), starlings were significantly less sensitive than all others to acute intoxication, and red-winged blackbirds (and probably house sparrows) were significantly more sensitive. If one considers each chemical group separately, these conclusions fail to hold for organochlorines. Red-winged blackbirds and starlings do not differ in their relative sensitivity to organochlorines; however, sparrows and coturnix do. The responses to carbamates and organophosphates match the overall response for all species.

Figure 8 presents the responses of starlings to the organochlorines in the previous data set. Starlings respond to organochlorines in a much different manner than they do to carbamates or organophosphates. Figure 9 shows data for the same chemicals for house sparrows; although sparrows appeared to respond similarly to organophosphates and carbamates but not organochlorines in Figure 7, they actually respond differently to all three chemical groups. Sparrows are highly responsive to organochlorines, moderately responsive to organophosphates, and much less responsive to

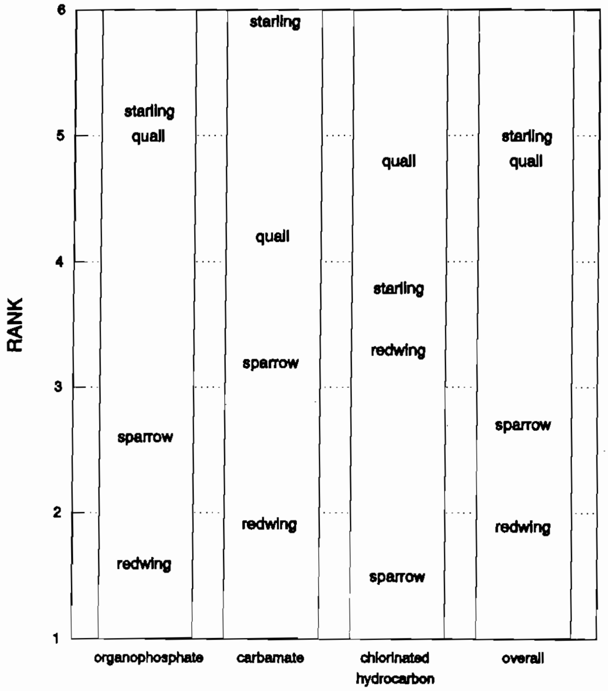

FIGURE 7. Comparison of ranking the acute oral toxicity sensitivity of four species of birds to three types of pesticides (1 = most toxic, 6 = least toxic).

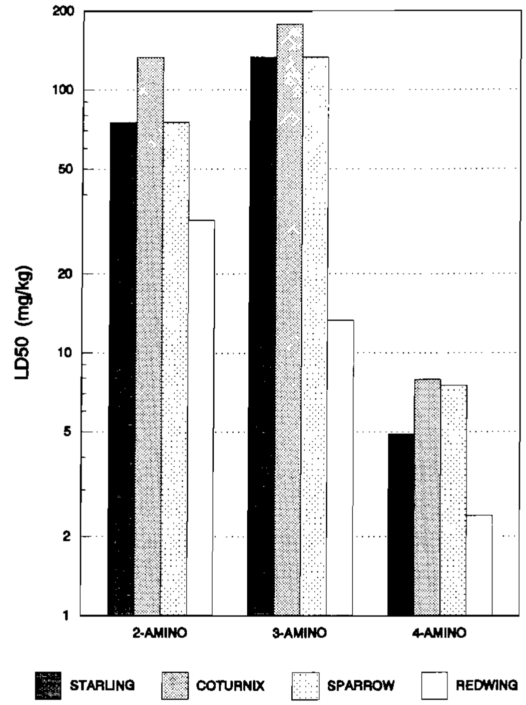

FIGURE 11. Acute oral toxicity of 2-, 3-, and 4-pyridineamines to four species of birds.

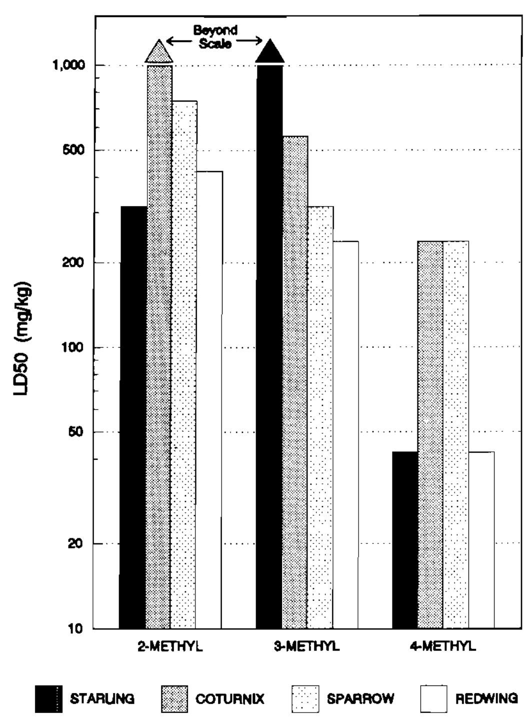

FIGURE 12. Acute oral toxicity of 2-, 3-, and 4-methylbenzenamines to four species of birds.

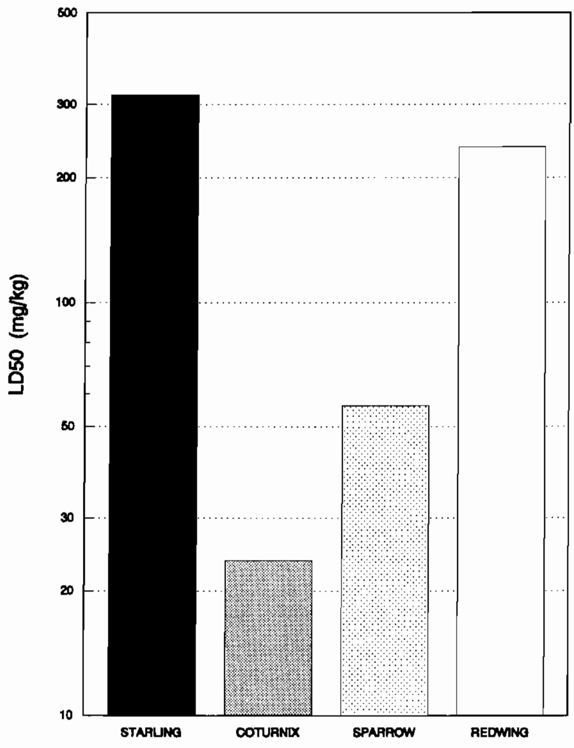

FIGURE 13. Acute oral toxicity of 4-aminobenzonitrile to four species of birds.

sensitivity to all the chemicals tested, and red-winged blackbirds were the most sensitive. It was also shown that for monosubstituted anilines across all species of birds, isomers were more toxic when proceeding structurally from the 2 to the 3 to the 4 isomer, and that the order of toxicity for monosubstituted pyridines increased from the 3 isomer to the 2 isomer to the 4 isomer. Although many of the data groups confirmed this general finding, there were numerous exceptions. For example, Figure 11 shows the results of testing three pyridineamine isomers. For these three chemicals, the 2-amino isomer is more toxic to three of the four species than the 3-amino isomer, but for one species (red-winged blackbird) the 3-amino isomer is more toxic than the 2-amino isomer. Although starlings were generally less sensitive to pyridineamine isomers than the three other species, coturnix are less sensitive than starlings; sparrows and starlings have a similar sensitivity compared to the more typical pairing of sparrows and coturnix. Although these differences are not large, they could be important if predictions of toxicity of a single isomer were being based on toxicity of another isomer. Figure 12 shows a similar situation of sensitivity/species reversals for methylbenzenamines. In this case, starlings are the most susceptible to intoxication from the 2-methyl isomer rather than the 3-methyl isomer, and starlings are similarly sensitive to intoxication by the 4-methyl isomer as are red-winged blackbirds. Figure 13 shows a similar reversal in species sensitivity to 4-aminobenzonitrile. In this case, if red-winged blackbirds were assumed to be the most sensitive species, toxicity estimates for coturnix and sparrows based on red-winged blackbird data could be off by at least one order of magnitude. Thus, the assumption about similar sensitivity of species to intoxication by isomers of chemicals within specific groups could be off by at least one order of magnitude.

RECOMMENDATIONS

At this time when producers, users, and regulators of chemicals are being asked to refine and improve their predictions of environmental risk, the tools available to improve the accuracy of models, to better define toxicological relationships, and to establish a basic understanding of the toxicological response of birds to introduced chemicals are further from their grasp. The accuracy of models is only as accurate as the information that goes into the models; if that information is incomplete or flawed, then the results will also be incomplete or flawed. Toxicologists need to address four questions that impact the future of comparative avian toxicology:

1. How good an estimate of potential environmental risk is acceptable and practical?
2. How can the scientific community access the limited amount of comparative avian toxicity data on wild bird species and use those data to support risk assessments, even though the data were not gathered under Good Laboratory Practice standards?
3. How can tests that were developed to generate comparative toxicity data be used for predictions on a chemical by chemical basis?
4. What do all these data mean when applied to the field and can laboratory tests be validated in the field?

These issues will become more critical with the public's increasing desire to be more informed on environmental issues and to minimize the environmental impact of chemicals. Perhaps it is time to qualify risk assessments by acknowledging that they give only crude, "ballpark" estimates and recognize the need for a type of directed post-registration monitoring to uncover "real" environmental risks.

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